TITLE PAGE

Protocol Title: An open label parallel group study to investigate the optimum methodology for the use of LPS or GM-CSF as challenge agents on healthy participants by assessing inflammatory biomarkers in cantharidin-induced skin blisters, peripheral blood, and urine.

Protocol Number: 207654 /01

Short Title: Skin blisters with systemic LPS or GM-CSF challenge

Compound Number: None

Sponsor Name and Legal Registered Address:

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Regulatory Agency Identifying Number(s): N/A

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SPONSOR SIGNATORY:

PPD

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7/AUG/2017
Date

VP Medicine Development Leader

PPD

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 1	*07-Aug-2017*
Original Protocol	*08-May-2017*

Amendment 1 07-Aug-2017

Overall Rationale for the Amendment: Change of GM-CSF (LEUKINE) administration from subcutaneous to IV infusion. This is due to liquid LEUKINE no longer being available from the manufacturer Sanofi-Aventis, however lyophilised LEUKINE is available. The guidelines for reconstitution of lyophilised LEUKINE prevent us from administering by subcutaneous as the volume would be too high. In order to remain consistent with clinical practice in dosing GM-CSF therapeutically the dose will be calculated using body surface area.

Section # and Name	Description of Change	Brief Rationale
1 Synopsis	Clarification to primary objective to remove the wording “in cantharidin-induced skin blisters”	To clarify that the primary objective was based on blood and urine samples.
2 Schedule of Activities	Removal of Session 2 Day-1 blood samples for mediators and flow cytometry	These were not required as the pre-challenge blood samples will be collected on Day 1
2 Schedule of Activities	Clarification of footnote for mediators blood sample time-points for pre challenge and pre blister	Given the change of administration of GM-CSF (LEUKINE) from subcutaneous to IV infusion the minus 30 minutes pre-blister induction was changed to pre challenge agent. The minus 5 minute time-point was clarified to mean pre-blister induction.
2 Schedule of Activities	Removal of injection site inspection assessment.	GM-CSF dose now being given as an IV infusion and no longer as an injection and therefore not required.
2 Schedule of Activities	Clarification of footnote for flow cytometry sample time-points and addition of a 9hour 40 minutes (10 hours post end of GM-CSF infusion) time-point for the GM-CSF group only.	Given the change of administration of GM-CSF (LEUKINE) from subcutaneous to IV infusion over 2 hours the minus 30 minutes pre-blister induction was no longer valid and so was changed to say pre challenge agent. The minus 5 minute time-point was clarified to mean pre-blister induction. The 9hour 40minutes post blister induction time-point was added as it is anticipated that the peak leukocyte count will occur between 8-12 hours and this will be required to make an accurate decision at dose escalation.
2 Schedule of Activities	Session 2 Day 2 urine collection time-point removed and footnote 12 moved to Day 1.	This was an error in the previous protocol as the urine collection was only until 12 hours post challenge agent.
4 Objectives and Endpoints	Clarification to primary objective to remove the wording “in cantharidin-induced skin blisters”	To clarify that the primary objective was based on blood and urine samples.
5 Study Design	Change of dose for GM-CSF in the study design schematic.	Given the change of administration of GM-CSF (LEUKINE) from subcutaneous to IV infusion the minimum and

Section # and Name	Description of Change	Brief Rationale
		maximum doses needed to change, as mentioned above.
5.1 Overall Design	Study design schematic session 2 changed to include pre-challenge blood draw instead of minus 30 minutes and 09:40 blood draw time-point added for GM-CSF only.	Given the change of administration of GM-CSF (LEUKINE) from subcutaneous to IV infusion over 2 hours the minus 30 minutes pre-blister induction was no longer valid.
5.5.3 GM-CSF (Dose Justification)	Initial dose amended and maximum dose amended. Removed the increments of 5µg/kg.	Initial dose amended to reflect change of GM-CSF administration from subcutaneous to IV infusion dose and to remain consistent with clinical practice in dosing GM-CSF therapeutically the dose will be calculated by body surface area. Increments of 5 µg/kg was now far too high with the change from subcutaneous injection to IV infusion and so the increments was removed.
7.1 Treatment Administration	GM-CSF section changed: liquid LEUKINE details removed. Dose changed, route of administration changed to IV infusion and dosing instructions amended.	Liquid Leukine no longer available to buy from manufacturer. Lyophilised LEUKINE is available and will be given as an IV infusion.
7.3.3 GM-CSF(Preparation/ Handling/ Storage/ Accountability)	Removal of dose injected as subcutaneous sentence.	IV infusion dose now being used.
9.3.3 Inspection of blister and injection sites	Removal of inspection of injection site	No longer required due to change in administration of GM-CSF.

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1. SYNOPSIS

Protocol Title: An open label parallel group study to investigate the optimum methodology for the use of LPS or GM-CSF as challenge agents on healthy participants by assessing inflammatory biomarkers in cantharidin-induced skin blisters, peripheral blood, and urine.

Short Title: Skin blisters with systemic LPS or GM-CSF challenge

Rationale: This study aims to assess 2 models of systemic inflammatory response: exposure of healthy participants to systemic challenge with either LPS or GM-CSF. This will be done by measuring inflammatory mediators and cellular activation markers both in circulation and in skin blisters induced by exposure to cantharidin (as a model of local inflammatory focus). More specifically, the study will assess the time course of up-regulation of circulating markers of inflammation (both cellular and soluble), in response to the systemic challenges and whether the challenges accentuate the inflammatory milieu of the blisters. The study will also assess urinary biomarkers of inflammation, and more specifically metabolites of prostaglandins, such as tetranor-PGDM. The literature reports that these stable urinary metabolites are elevated in patients with Duchenne muscular dystrophy and they are mechanistically relevant to treatments currently being evaluated at GSK. Data generated in this study will provide the foundation for future studies testing the effect of compounds that target key pathways in inflammation and autoimmunity.

Objectives and Endpoints:

Objective	Endpoint
Primary	
• To compare and define the time course of soluble and cellular circulating inflammatory biomarkers (and urinary prostaglandins for LPS only) following systemic challenge with LPS or GM-CSF in healthy participants	• For LPS only: time course and magnitude of upregulation of circulating TNF-α and IL-6 as well as urinary tetranor PGDM. • For GM-CSF only: time course and magnitude of upregulation of circulating total leukocyte numbers.

Secondary	
• To compare soluble and cellular inflammatory biomarkers in cantharidin-induced skin blisters at baseline versus systemic challenge with LPS or GM-CSF in healthy participants	• Soluble inflammatory biomarkers in skin blisters (may include but not limited to IL-1b, IL-2, IL-6, IL-8, IFNg, TNF-α, MCP-1, GM-CSF, CRP) • Blister volumes and differential cell counts (cellular activation markers may include, but not limited to expression of CD16, CD86, CD80, CD163, CD206, CD83, CD40, CD209, HLA-DR in blister leukocytes)
• To define the time course of circulating soluble and cellular inflammatory biomarker upregulation following systemic challenge with LPS or GM-CSF in healthy participants	• Time course of regulation of circulating soluble inflammatory biomarkers (may include but is not limited to IL-1b, IL-2, IL-6, IL-8, IFNg, TNF-α, MCP-1, GM-CSF, CRP) • Time course of regulation of circulating leukocyte numbers and cellular activation markers (may include, but not limited to expression of CD16, CD86, CD80, CD163, CD206, CD83, CD40, CD209, HLA-DR in circulating leukocytes)

Overall Design:

This is an exploratory parallel design study comprising of 2 parts. In both parts of the study, participants will have 2 sessions. Session 1 will include a blister induction followed by blood draws and a blister harvest on each forearm. After a minimum of 14 days to allow for blister healing, participants will return for Session 2 where they will receive either a LPS or GM-CSF *in vivo* challenge (4 completed participants in each group). Participants will then have another blister induction, followed by multiple, timed, blood draws and blister harvests.

For Part I, a dose exploration design is used to find a dose that provides a robust inflammatory response for both LPS and GM-CSF. Up to 6 cohorts of 4 participants will be tested and all cohorts will have 2 sessions, as described above. During this dose exploration phase the response to each challenge agent will be assessed by dose escalation meeting after 2 participants have been exposed to that agent. The response to

LPS will be primarily assessed based on IL-6, TNF-α and urinary tetranor PGDM. The response to GM-CSF will primarily be based on leukocyte counts. The decision whether to adjust the dose will be made by the Principal Investigator in consultation with the medical monitor based on the response outlined above and in the context of the safety and tolerability at that dose.

For Part II, an additional cohort of up to 8 participants may be enrolled if an interim analysis indicates that this would provide further precision on estimates of primary endpoints. The same 2-session design, as described above, would be used and participants will be dosed with LPS and GM-CSF at the same dose as the 8 evaluable participants from Part I .

Number of Participants: It is estimated that up to 8 participants will be evaluated at the optimal dose for each challenge agent. Accounting for dose-findings, an estimated 24-30 (maximum of 40, including replacement of any drop-outs) participants will be enrolled.

Treatment Groups and Duration: Participants will be randomised to receive either a LPS or GM-CSF *in vivo* challenge. The total study duration for each participant is approximately 13 weeks from screening to the final follow-up.

2. SCHEDULE OF ACTIVITIES (SOA)

Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files, but will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

PROCEDURE	**Screening**	**SESSION 1**			**Interim inspection**	**SESSION 2**				**1st follow-up**	**2nd follow-up**	
Day:	Within 30 days of Day 1	Day 1	Day 2	Day 3	Minimum 2 wks (max 4 weeks) after end of session 1	Day -1	Day 1	Day 2	Day 3	Minimum 2 wks (max. 4 weeks) after end of session 2	Approx. 5wks after end of session 2	
Attend Unit	X	X	X	X	X	X[3]	X[3]	X	X	X	X	1. Brief physical exam only
Informed Consent	X											2. Within an hour before cantharidin application
Inclusion/Exclusion checklist	X					X						3. Overnight stay in the unit
Medical /medication history	X											4. Additional clinical lab assessments may be performed, if necessary
Demographics	X											5. Before blister fluid sampling
Body weight	X					X						6. After the last blister fluid sampling at each session
Drug/alcohol test	X	X				X						7. Multiple blood draws at the following time points: Pre challenge, , 5 mins (i.e. Pre-blister induction), 10 min, 25 min, 40 min, 1hr:10mins, 1hr:40mins, 2hr:40mins, 5hr:40mins with respect to start of blister induction
Physical Exam	X	X[1]				X	X[1]			X[1]		
Vital Signs and temperature	X	X[2]				X	X[8]	X		X	X	
ECG	X					X	X					
Telemetry							X					
Visual forearm check (including blister healing and cosmetic assessment)	X	X[2]			X	X	X[2]			X	X	
Cantharidin application (Session 2 only: 20 minutes post end of LPS or GM-CSF challenge)		X					X					
AE assessment/Con Meds		«----------									----------»	8. Every half hour for the first 4 hours after challenge, hourly until 8 hours and then 8 hourly until discharge. Frequency can be increased if symptomatic
SAE	«----------										----------»	
Clinical Chemistry, Haematology, and Urinalysis [4]	X	X	X	X		X	X	X	X			
Mediators blood sample		X[2]	X[5]	X[5]			X[7]	X[5]	X[5]			9. For flow cytometry and transcriptomic blood samples: Multiple blood draws at the
Flow cytometry blood sample		X[2]	X[5]	X[5]			X[9]	X[5]	X[5]			
For LPS only: *Ex vivo*							X[10]					

PROCEDURE	**Screening**	**SESSION 1**			**Interim inspection**	**SESSION 2**				**1st follow-up**	**2nd follow-up**
Day:	Within 30 days of Day 1	Day 1	Day 2	Day 3	Minimum 2 wks (max 4 weeks) after end of session 1	Day -1	Day 1	Day 2	Day 3	Minimum 2 wks (max. 4 weeks) after end of session 2	Approx. 5wks after end of session 2
stimulation blood sample											
Transcriptomic blood sample							X[9]				
Urine sampling for PD			X[11]				X[11]				
For LPS only: Intravenous hydration with normal Saline at a rate of 250 mL / hr							X[12]				
In vivo LPS or GM-CSF challenge							X[2]				
Blister sample for biomarkers			X	X				X	X		
Pain rating[6]				X					X		
Participant diary card given to participant to record blister healing		X					X				

following time points: Pre challenge, 40 mins, 2hr:40mins and 5hr:40mins (and 9hr 40mins for GM-CSF for flow cytometry only) with respect to start of blister induction

10. *Ex vivo* stimulation blood sample: 2 samples (one null and one LPS tube) to be taken pre-dose and 2 samples (one null and one LPS tube) to be taken at 5hrs:40mins post blister induction
11. Pre-challenge urine sample will be collected in session 1. For the post-challenge samples in session 2, participants will be encouraged to pass urine immediately before LPS dosing and each urine void will be collected after LPS until 12 hours post-LPS
12. From 4 hours prior to LPS until 8 hours after LPS

3. INTRODUCTION

3.1. Cantharidin-induced skin blister assay

The cantharidin blister model was chosen to permit easy access to an acute in vivo inflammatory compartment, in which response to inflammatory challenges might be investigated directly by analysing cellular and soluble components.

Cantharidin is a vesicant (an agent that causes blisters) and a strong inhibitor of protein phosphatases type 1 and 2A (Honkanen, 1993). Following contact with the skin, it is absorbed into the epidermis and activates neutral serine proteases that cause dissolution of desmosomes, leading to acantholysis (the disruption of intercellular bridges between keratinocytes) and intra-epidermal blistering (Bertaux, 1988). The pathology is limited to the suprabasal epidermis and lesions heal without scarring (Moed, 2001). Topical cantharidin solution (0.7%) is used in clinical dermatological practice in Canada for the treatment of warts and molluscum contagiosum and has been demonstrated as an effective treatment, including in children (Noda-Cabrera, 2015). Skin blister induction by cantharidin has been utilised in clinical pharmacology for more than 50 years either as a tool for pharmacokinetic studies or as a model of acute inflammation in human participants. The blister fluid formed after 24 hours of exposure to cantharidin contains inflammatory cells, predominantly neutrophils and monocytes/macrophages, that may be counted (to evaluate leukocyte trafficking and cellular accumulation), analysed by flow cytometry (to perform phenotypic profiling), or used for ex-vivo assays. In parallel, various cytokines, chemokines and other mediators of inflammation may be measured in blister fluid (Day, 2001, Evans, 2006, Harbord, 2006, Evans, 2008). It has been demonstrated that the inflammatory response measured in the cantharidin-induced blister fluid is sensitive to pharmacological manipulation using anti-inflammatory treatment (Morris, 2009). Furthermore, prolonged exposure to cantharidin (40+ hours) allows insight into the resolving phase of acute inflammation (Yagnik, 2000, Philippidis, 2004). In healthy volunteers this phase includes polarisation of monocyte-derived macrophages largely towards a resolving phenotype (Yagnik, 2000, Day, 2001), with enhanced expression of markers such as CD163 and CD206 (Philippidis, 2004) GlaxoSmithKline Document Number (GM2008/00294/00); GlaxoSmithKline Document Number (AM2010/00011/01).

3.2. Study Rationale

This study aims to assess 2 models of systemic inflammatory response: exposure of healthy participants to systemic challenge with either LPS or GM-CSF. This will be done by measuring inflammatory mediators and cellular activation markers both in circulation and in skin blisters induced by exposure to cantharidin (as a model of local inflammatory focus). More specifically, the study will assess the time course of up-regulation of circulating markers of inflammation (both cellular and soluble), in response to the systemic challenges and whether the challenges accentuate the inflammatory milieu of the blisters. The study will also assess urinary biomarkers of inflammation, and more specifically metabolites of prostaglandins, such as tetranor-PGDM. The literature reports that these stable urinary metabolites are elevated in patients with Duchenne muscular dystrophy and they are mechanistically relevant to treatments currently being evaluated at

GSK. Data generated in this study will provide the foundation for future studies testing the effect of compounds that target key pathways in inflammation and autoimmunity.

LPS is often used to induce inflammation to model disease in animal models and in *in vitro* systems. Human *in vivo* LPS challenges have been used as a methodology to induce systemic inflammation and produce many of the immunological (changes in leukocyte numbers and induction of inflammatory mediators) and physical signs of acute and chronic disease (including fever, pain, and tachycardia). The human LPS model of systemic inflammation has been applied to clinical pharmacology studies to assess therapeutic interventions for analgesics, asthma, effective adjuvants, sepsis, trauma, Type-2 diabetes, Alzheimer's disease and others. This study will characterize the phenotype of peripheral leukocytes and the production of a plethora of inflammatory mediators and acute phase proteins over a time course. With regards to prostaglandins, this pathway has been reported in the literature to be disregulated in patients with Duchenne muscular dystrophy (Nakagawa, 2013). However, in normal healthy individuals, these inflammatory mediators and their metabolites have very low circulating and urine concentrations, respectively. Therefore, artificial stimulation of this pathway by LPS in normal healthy volunteers would be required for early clinical evaluations of target engagement for therapeutics target this pathway.

GM-CSF has a broad range of activities across innate and adaptive immune cells and is recognised as a key mediator in a number of inflammatory diseases, such as arthritis, multiple sclerosis, colitis, pain and interstitial lung disease (Wicks, 2016). 5 µg / kg Leukine (Sargramostim) given subcutaneously in the abdominal area results in a systemic GM-CSF challenge that mobilises neutrophils, eosinophils and monocytes from the bone marrow. Changes in leukocyte numbers have previously been characterised (van Pelt, 1996) with only minor efforts in detecting secreted inflammatory mediators. This study will extend these observations over a defined time course to include a plethora of soluble inflammatory mediators as well as phenotype peripheral leukocytes, particularly monocytes.

3.3. Background

3.3.1. LPS challenge

Endotoxin lipopolysaccharide (LPS) is a major component of the outer membrane of Gram negative bacteria. It is one of the pathogen-associated molecular patterns that will trigger inflammation by the binding to pattern recognition receptors (PRRs), initiating downstream intracellular signalling pathways that result in the activation of the nuclear transcription factor, nuclear factor kB (NF-kB) (Zhang, 2001), which in turn stimulates the transcription of genes coding for pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-1β (May, 1998). These cytokines activate an assortment of inflammatory cascades including the complement system, the coagulation system and the production of nitric oxide, all of which participate in eliminating invading microorganisms (Janeway, 2002).

Systemic inflammation is a pathogenic component in a vast number of acute and chronic diseases such as sepsis, trauma, type 2 diabetes, atherosclerosis, and Alzheimer's disease,

all of which are associated with a substantial morbidity and mortality. Human models of systemic inflammation have been developed with the purpose of mimicking the changes in inflammatory mediators encountered in acute as well as chronic inflammatory disease, but in a controlled, standardised experimental setting. These also allow the study of the molecular mechanisms and physiological significance of the systemic inflammatory response. The most widespread model of systemic inflammation is probably the human endotoxin model, in which purified LPS from *Escherichia coli* or other Gram-negative bacteria is administered intravenously to healthy volunteers, inducing an acute systemic inflammatory response, which, at least partially, mimics the inflammatory response of early sepsis as well as other acute inflammatory conditions (Andreasen, 2008).

After injection, LPS can be measured in plasma within a few minutes, and is quickly transferred (plasma levels drop sharply within the next 15 minutes) to the liver to be metabolised (van Deventer, 1990). Usually within one hour of LPS administration, volunteers will experience varying degrees of flu-like symptoms (e.g. headache, chills, myalgia and nausea), with the symptoms slowly disappearing after 4 to 6 hours, in parallel with the diminishing inflammatory response. The most reproducible clinical findings among participants include an increase in core temperature and tachycardia (Dinarello, 2004).

Administration of LPS causes a quick decline in neutrophil numbers in circulation in the first 15-30 minutes, probably from the margination of these cells due to upregulation of vascular adhesion factors, followed by a 3-4 fold increase in the following 4 to 6 hours and a return to baseline within 24 hours after exposure (Richardson, 1989, van Deventer, 1990, Jilma, 1999). At the same time, monocytopenia and lymphopenia are observed upon LPS challenge with monocytes having a more rapid decline (trough at around 90 minutes) and return to baseline (6-8 hour) (Thaler, 2016) whereas lymphocyte counts are most reduced at 4 hours and only normalise after 8-12 hours (Krabbe, 2001). Monocyte subsets are differentially affected by LPS. After 24 hours, distribution was skewed towards the intermediate (CD16+ CD14+) subset. These monocytes also displayed the largest increase of CD11b expression after 2 hours and the highest increase in IL-6 and IL-8 mRNA levels, whereas these mRNA levels in classical monocytes (CD14+ CD16-) change only marginally. (Thaler, 2016).

3.3.2. GM-CSF Challenge

Granulocyte–macrophage colony-stimulating factor (GM-CSF) is a cytokine, belonging to the colony-stimulating factors (CSF), crucial for survival, proliferation, differentiation, maturation and functional activation of haematopoietic cells. It is often used to treat leukopenia, but as other haematopoietins may increase the number of circulating leukocytes with higher efficiency, GM-CSF has additional effects that may be far more relevant than its haematopoietic activity. It induces differentiation, proliferation and activation of macrophages and dendritic cells which are necessary for subsequent T helper cell type 1 and cytotoxic T lymphocyte activation (Francisco-Cruz, Aguilar-Santelises et al. 2014).

GM-CSF is used in the clinical setting for the treatment of bone marrow dysfunction. Healthy donors may be stimulated with the growth factor to induce generation of

peripheral blood progenitor cells (PBPC) or granulocyte for donation. (Fischmeister, 1999)

3.3.3. Pharmacokinetics and pharmacodynamics of GM-CSF

When GM-CSF (also known as sargramostim) is administered to patients by intravenous infusion over 2 hours, it reaches a mean beta half-life of approximately 60 minutes. The peak concentrations of GM-CSF are observed in blood samples obtained during or immediately after completion of the sargramostim infusion, while minor concentrations are still detected in blood 6 hours after the beginning of the infusion. GM-CSF is also detected in serum first after 15 minutes of subcutaneous injection of sargramostim to healthy volunteers. Then, the mean beta half-life is approximately 162 minutes, and peak levels are reached after one to 3 hours post-injection and remain detectable for up to 6 hours.

Sargramostim is usually well tolerated by healthy participants, who have not shown clinical alterations in their clinical analysis, as compared to placebo-treated individuals. (Francisco-Cruz, 2014)

3.4. Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of cantharidin may be found in the Participant Information Leaflet.

3.4.1. Risk Assessment

The challenge agents and cantharidin used in this study are established in healthy volunteer studies and are expected to be well tolerated. The information in this section is provided in the interests of full disclosure of all potential risks.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Cantharidin		
Risk of lymphangitis and/or Lymphoedema	Three cases of lymphangitis and lymphoedema following high exposure to cantharidin (0.7% solution and application on multiple warts) have been described in the literature (Dilaimy, 1975, Stazzone, 1998).	Participants with a history of lymphangitis and/or lymphoedema or any participant who has undergone surgery resulting in loss of tissue associated with lymphoid drainage (e.g. certain breast surgery procedures) are excluded. Concentration of cantharidin is reduced in this study from 0.7% to 0.2 %.
Discomfort and/or pain at the blister site	Forearm blister induction with cantharidin has been associated with local discomfort and/or pain in previous studies.	Participants are advised to wear loose clothing at the site of blistering to minimise hyperaesthesia and discomfort that may occur. Pain will be assessed by asking participants to rate their pain using an 11-point scale (0 – no pain to 10 –worst pain imaginable) (Farrar, 2001). For immediate relief of any discomfort, paracetamol, at doses of $\leq$ 2 grams/day will be given to the participant, unless, in the opinion of the investigator and sponsor, the medication will interfere with the study.
Risk of dyspigmentation and hypertrophic scar	After blister healing, temporary post-	Participants with history of keloids, skin allergy,

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
formation	inflammatory dyspigmentation and hypertrophic scar may occur and persist for several months.	hypersensitivity, or contact dermatitis, including previous reactions to dressings to be used in this study or any chronic skin disorder (e.g. psoriasis, atopic dermatitis, vitiligo) excepting isolated lesions remote from intended site of application of cantharidin are excluded. Appropriate skin care to avoid dyspigmentation and hypertrophic scar will be implemented.
Risk of infection at the blister site	During the blister fluid harvesting, there is potential for infection at the site. To date there are no reports of skin infection from clinical studies evaluating cantharidin-induced skin blister model.	To minimize the risk, appropriate clean procedures will be applied. Participants are instructed to visually inspect blister sites on a daily basis and are informed what signs of inflammation/infection to watch for and contact the investigator with any concerns.
Accidental cantharidin ingestion or contact with eyes and mucus membranes	Fatal poisoning may occur if cantharidin is swallowed. However, no cases caused by application of cantharidin solution by a physician (or supervised by) have been reported to date. There are also no reports of accidental contact with eyes or mucus membranes from experimental use of cantharidin.	Cantharidin will not be dispensed to participants and all applications will be done at the research centre by qualified personnel.
LPS		
Risk of exaggerated physiological response to intravenous LPS	Intravenous LPS can cause short-lived inflammatory response in healthy volunteers. Previous studies have shown that single intravenous doses of 0.6ng/kg LPS were well tolerated in healthy male participants. Observed	The dose of LPS in this study will be carefully titrated to identify a dose that is associated with a robust inflammatory response but is well tolerated. The administered dose will be informed by previous published clinical

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	adverse events (AEs) were of mild severity and self-limiting without therapeutic intervention. The most frequent occurring AEs were headache, and feeling cold. No clinically relevant changes or unexpected treatment-related trends were observed in supine systolic and diastolic blood pressure, body temperature, or ECG-derived parameters following administration of LPS. LPS can dose-dependently increase body temperature and heart rate, with a maximal increase amounting approximately 1.5 °C and 28 ± 13.2 bpm for LPS dose of 2 ng/kg (10 EU/ng) tested, observed at 3–4 hours after LPS administration (Dillingh, 2014).	experience with LPS and the in vitro activity of the actual batch of LPS to be given. During the dose finding phase of the study only one participant will be dosed at a time with LPS. The participants will be dosed with LPS in a MHRA accredited clinical research unit and will have regular monitoring of vital signs post dose. The frequency of monitoring can be increased if there are clinical concerns. The dosed participants will be confined to clinical unit for approx. 24 hours post dosing with LPS. The age range for participants in this study is restricted to 18-45 years old to minimise the possibility of exaggerated response to LPS. Participants will receive telemetry for a minimum of 6 hours post-dose or until their telemetry shows no clinical significant findings for 4 hours (whichever is longer). Participants will be prehydrated with intravenous fluids prior to LPS challenge, and intravenous fluid hydration will continue following the challenge. Normal saline will be infused at a rate of 250ml/hr for 4 hours prior to LPS dosing and 8 hours subsequently.
Risk of severe vagal response	IV LPS endotoxin challenge has been administered in the higher dose range of 2 - 4 ng/kg (10 EU/ng) to thousands of individuals with	Participants with previous history of vasovagal syncope will be excluded from the study. Participants will be pre-hydrated with intravenous

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	no serious or permanent adverse events (Fullerton, 2016). However isolated accounts of severe vagal reactions have been reported (van Eijk, 2004). The aetiology presumably represents high resting vagal tone, volume depletion after overnight fasting, and catecholamine release with the onset of fever, chills and symptoms, culminating in an exaggerated Bezold-Jarisch reflex.	fluids prior to LPS challenge, and intravenous fluid hydration will continue following the challenge. Normal saline will be infused at a rate of 250ml/hour for 4 hours prior to LPS dosing and 8 hours subsequently.
Endotoxin tolerance	It has been shown that endotoxin tolerance may persist in some individuals for an unknown length of time *in vivo*. (Draisma, 2008, Kox, 2011)	Participants with previous experimental exposure to LPS will be excluded.
GM-CSF		
Risk of Fluid Retention	Oedema, capillary leak syndrome, pleural and/or pericardial effusion have been reported in patients after LEUKINE administration. In 156 patients enrolled in placebo-controlled studies using LEUKINE at a dose of 250 mcg/m2/day by 2-hour IV infusion, the reported incidences of fluid retention (LEUKINE vs. placebo) were as follows: peripheral oedema, 11% vs. 7%; pleural effusion, 1% vs. 0%; and pericardial effusion, 4% vs. 1%. Capillary leak syndrome was not observed in this limited number of studies; based on other uncontrolled studies and reports from users of marketed LEUKINE, the incidence is estimated to be less than 1%. In patients with pre-existing pleural and pericardial effusions, administration of LEUKINE may aggravate fluid	Use of healthy volunteers is a mitigation as these findings were observed in patients with cancer in receipt of chemotherapy. A lower, single dose of GM-CSF is planned which means that the AUC and Cmax will be lower than in the patients in which this was observed.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	retention; however, fluid retention associated with or worsened by LEUKINE has been reversible after interruption or dose reduction of LEUKINE with or without diuretic therapy. LEUKINE should be used with caution in patients with pre-existing fluid retention, pulmonary infiltrates or congestive heart failure.	
Risk of Respiratory Symptoms	Sequestration of granulocytes in the pulmonary circulation has been documented following LEUKINE infusion12 and dyspnoea has been reported occasionally in patients treated with LEUKINE. Special attention should be given to respiratory symptoms during or immediately following LEUKINE infusion, especially in patients with pre-existing lung disease. In patients displaying dyspnoea during LEUKINE administration, the rate of infusion should be reduced by half. If respiratory symptoms worsen despite infusion rate reduction, the infusion should be discontinued. Subsequent IV infusions may be administered following the standard dose schedule with careful monitoring. LEUKINE should be administered with caution in patients with hypoxia.	Patients with known respiratory disease will be excluded from the study (as described in the Exclusion criteria), as well as patients with current or recent (<30 days) infection.
Risk of cardiovascular Symptoms	Occasional transient supraventricular arrhythmia has been reported in uncontrolled studies during LEUKINE administration, particularly in patients with a previous history of cardiac arrhythmia. However, these arrhythmias have been reversible after discontinuation of LEUKINE.	Patients with pre-existing cardiac disease will not be eligible to participate. In addition, we will use telemetry for a minimum of 6 hours following dosing, or until normal for 4 hours (whichever is longer).

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	LEUKINE should be used with caution in patients with pre-existing cardiac disease.	
Risk of renal and Hepatic Dysfunction	In some patients with pre-existing renal or hepatic dysfunction enrolled in uncontrolled clinical trials, administration of LEUKINE has induced elevation of serum creatinine or bilirubin and hepatic enzymes. Dose reduction or interruption of LEUKINE administration has resulted in a decrease to pre-treatment values. However, in controlled clinical trials the incidences of renal and hepatic dysfunction were comparable between LEUKINE (250 mcg/m2/day by 2-hour IV infusion) and placebo-treated patients. Monitoring of renal and hepatic function in patients displaying renal or hepatic dysfunction prior to initiation of treatment is recommended at least every other week during LEUKINE administration.	Patients with pre-existing renal or hepatic dysfunction will be excluded from the study.

3.4.2. Benefit Assessment

There will be no benefit to the participant taking part in this study. However, participants will be contributing to the process of developing new therapies by taking part in the study.

3.4.3. Overall Benefit : Risk Conclusion

The measures taken to minimize the potential risks identified in association with cantharidin, LPS and GM-CSF are considered sufficient to justify participation by healthy volunteer participants.

4. OBJECTIVES AND ENDPOINTS

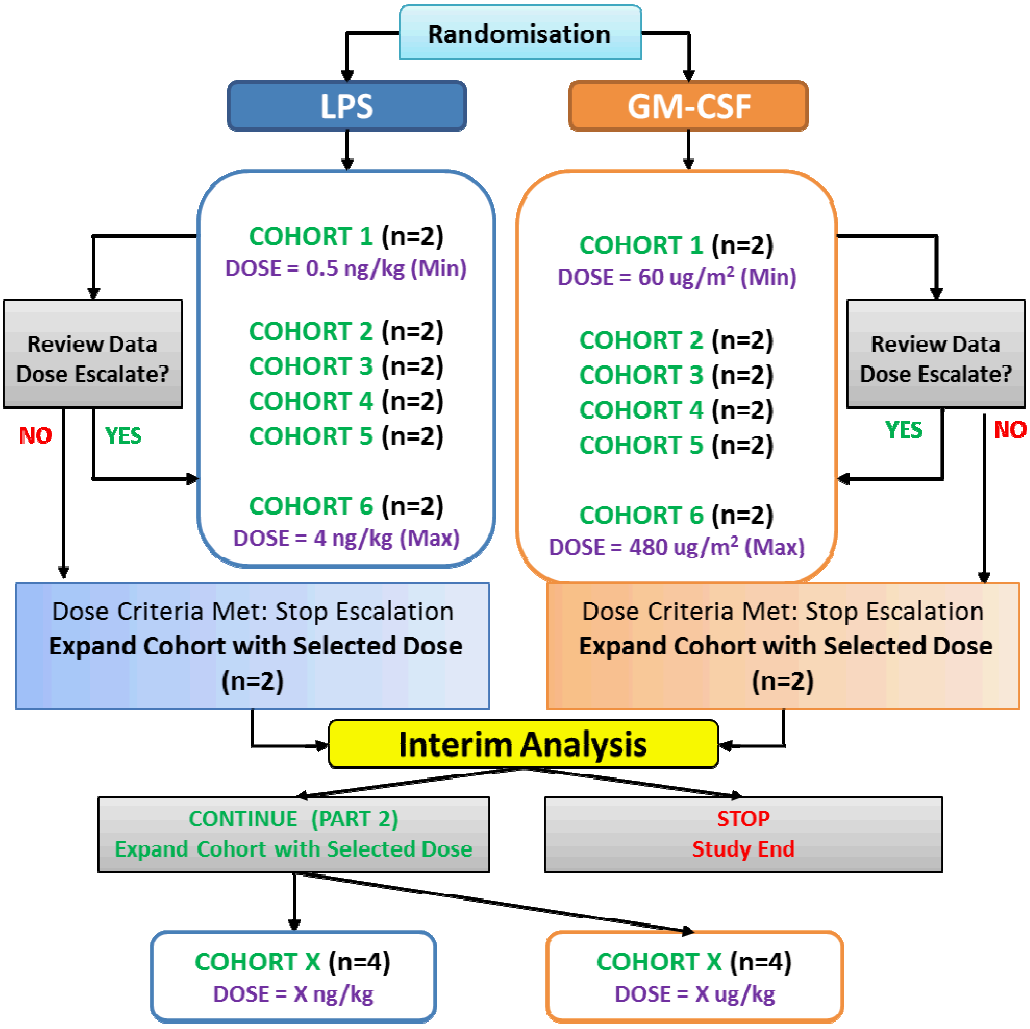
Objective	Endpoint
Primary	
• To compare and define the time course of soluble and cellular inflammatory biomarkers (and urinary prostaglandins for LPS only) following systemic challenge with LPS or GM-CSF in healthy participants	• For LPS only: time course and magnitude of upregulation of circulating TNF-α and IL-6 as well as urinary tetranor PGDM. • For GM-CSF only: time course and magnitude of upregulation of circulating total leukocyte numbers
Secondary	
• To compare soluble and cellular inflammatory biomarkers in cantharidin-induced skin blisters at baseline versus systemic challenge with LPS or GM-CSF in healthy participants	• Soluble inflammatory biomarkers in skin blisters (may include but not limited to IL-1b, IL-2, IL-6, IL-8, IFNg, TNF-α, MCP-1, GM-CSF, CRP). • Blister volumes and differential cell counts (cellular activation markers may include, but not limited to expression of CD16, CD86, CD80, CD163, CD206, CD83, CD40, CD209, HLA-DR in blister leukocytes)

Objective	Endpoint
• To define the time course of circulating soluble and cellular inflammatory biomarker upregulation following systemic challenge with LPS or GM-CSF in healthy participants	• Time course of regulation of circulating soluble inflammatory biomarkers (may include but is not limited to IL-1b, IL-2, IL-6, IL-8, IFNg, TNF-α, MCP-1, GM-CSF, CRP). • Time course of regulation of circulating leukocyte numbers and cellular activation markers (may include, but not limited to expression of CD16, CD86, CD80, CD163, CD206, CD83, CD40, CD209, HLA-DR in circulating leukocytes)
Exploratory	
• To compare suitability of blisters sampled at 24 and 48 hours after induction for maximising observed effects of systemic challenge	• Primary and Secondary endpoints measured in 24 versus 48 hour blisters
• To assess the safety and tolerability profile of the challenge agents and to ensure it is not materially different from previous experience (as described in Section 3.2)	• Adverse events, clinical laboratory measures, vital signs, pain scale assessments
• Determining whether carboxyesterase-1 (CES-1) expression is present in monocytes following systemic GM-CSF challenge	• Number of monocytes which are CES-1+ in blood
• To compare prostaglandins in urine, blister fluid and plasma at baseline versus systemic challenge with LPS or GM-CSF	• Quantification of prostaglandins including (but not limited to) tetranor -PGDM, tetranor-PGEM, PGD2 and PGE2
• To assess changes in whole blood transcriptome induced by systemic LPS- or GM-CSF-challenge	• Analysis of differential gene expression in whole blood taken pre-challenge and at selected times post- challenge

Objective	Endpoint
• For LPS challenge only: to assess whether the dose of *in* -vivo LPS challenge is sufficient to induce innate immune tolerance	• Quantification of inflammatory markers including (but not limited to) TNF-α and IL-6 in blood drawn pre- and 6 hour post-systemic LPS challenge following ex vivo incubation in 'LPS-TruCulture' tubes and LPS-null TruCulture' tubes
• To assess the effects of systemic LPS and GM-CSF challenges on blister healing times	• Blister healing times (self-reported in participant diary card)

5. STUDY DESIGN

Figure 1 Study design schematic



5.1. Overall Design

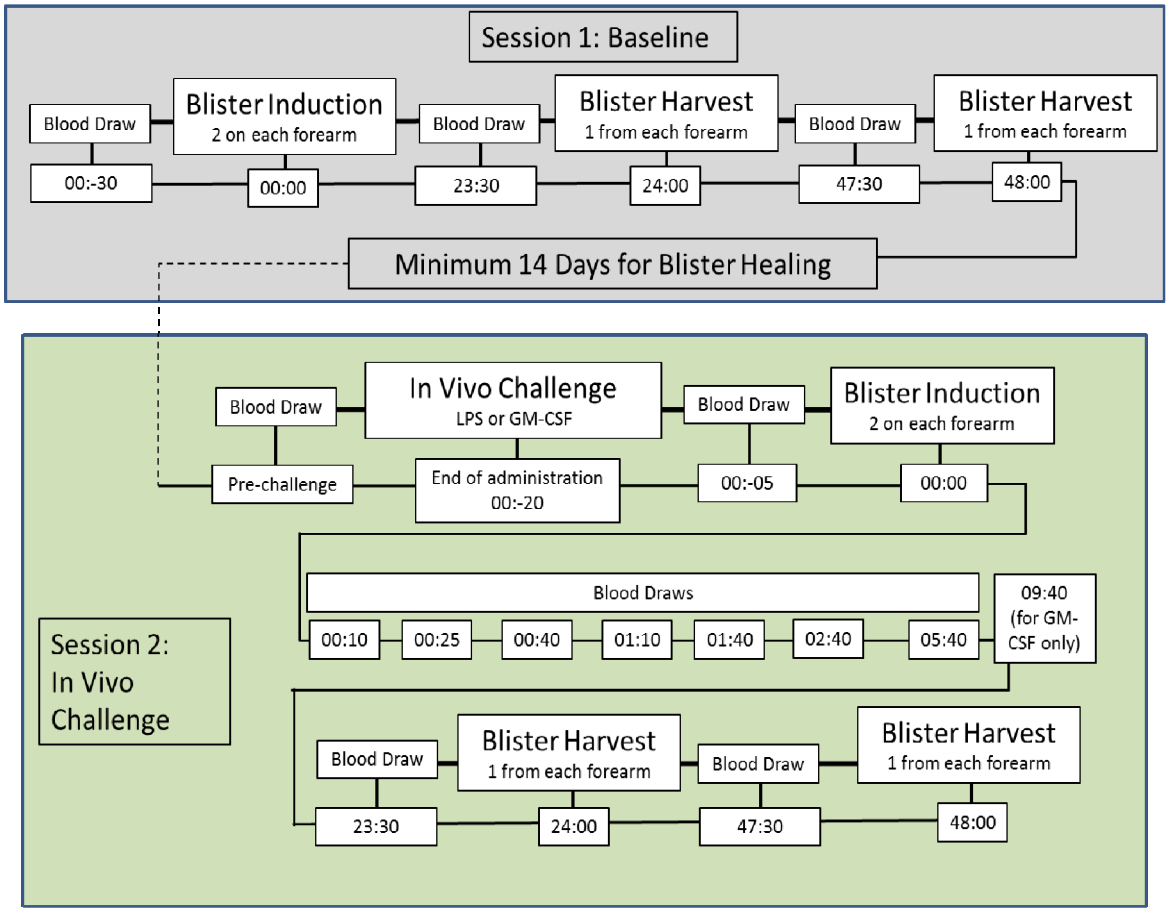
This is an exploratory study comprising of 2 parts. In both parts of the study, participants will have 2 sessions. In session 1 (baseline) all participants will have 2 blisters induced on each forearm by application of cantharidin solution (based on method described by Day et al. (Day, 2001)) followed by blood draws and a blister harvest on each forearm at 24 and 48 hours post-induction. After a minimum of 14 days blister healing period, participants will return for a second session (*in vivo* challenge session). This open label study will randomise participants to have either a LPS or GM-CSF *in vivo* challenge (4 completed participants in each group). Participants will then have 2 blisters induced on each forearm by application of cantharidin solution, followed by multiple, timed, blood draws (15 minutes to 48 hours post dose) and a blister harvest on each forearm at 24 and 48 hours post-induction.

In part I of the study, a dose exploration of each of the challenge agents (LPS and GM-CSF) will be required to find a dose that provides a robust inflammatory response. Up to 6 cohorts of 4 participants will be tested and all cohorts will have 2 sessions, as described above. For Part I, an initial cohort of 4 participants (Cohort 1) will proceed with session 1. After their blister healing period, Cohort 1 will return for their session 2 visit in two groups of 2 participants (Group A and Group B) on different days. Group A will be dosed on the same day (one with LPS and one with GM-CSF) and Group B will be dosed on a different day (one participant with LPS and one participant with GM-CSF) after group A. Once all 4 participants have been dosed, a dose escalation meeting will be held to make an assessment of tolerability and inflammatory response from the first Cohort (e.g. the primary endpoints) to determine the dose for Cohort 2. This dose escalation meeting will be held prior to Group A in Cohort 2 proceeding to be dosed with the challenge agents in Session 2. This same dose escalation procedure will continue until a well-tolerated dose for LPS and GM-CSF showing a robust inflammatory response has been identified. Once the well-tolerated dose with robust inflammatory response has been determined, additional participants will be administered this same dose until there are 8 evaluable participants (4 with LPS at the same dose and 4 with GM-CSF at the same dose) completed in Part I.

During this dose exploration phase the response to each challenge agent will be assessed by dose escalation meeting after 2 participants have been exposed to that agent. The response to LPS will be primarily assessed based on IL-6, TNF-α and urinary tetranor PGDM. The response to GM-CSF will primarily be based on leukocyte counts.. The decision whether to adjust the dose will be made by the Principal Investigator in consultation with the medical monitor based on the response outlined above in the context of the safety and tolerability at that dose. During the dose escalation phase, samples collected for secondary and exploratory endpoints will only be processed if the assay is required to be done immediately. Otherwise, collected samples will be batch processed once the optimal dose for each challenge has been established.

For Part II, an additional cohort of up to 8 participants (4 participants with GM-CSF and 4 participants with LPS challenge) at the same dose as the 8 evaluable participants from Part I may be added if the interim analysis indicates additional participants would provide further precision on estimates of primary endpoints. The same two-session design, as described above, would be used.

Figure 2 Study Design schematic of session 1 and 2 for each participant



5.2. Number of Participants

In Part I, up to 8 evaluable participants will be required at the optimal dose (4 of each challenge agent). Following an interim analysis, a sample size re-estimation will be conducted and up to a further 8 evaluable participants (up to 4 in each arm of challenge agent) in Part II may be enrolled and dosed at the same dose as Part I. Accounting for dose escalation exploration, an estimated 24-30 (maximum of 40 including replacement for drop-outs) participants will be enrolled in total. If participants prematurely discontinue the study, additional replacement participants may be recruited and assigned to the same treatment sequence, if that participant has not completed session 2.

5.3. Participant and Study Completion

A participant is considered to have completed the study if he/she has completed up to the end of session 2 of the study. If a participant withdraws before the end of session 2 they will be encouraged to return for a follow-up visit as per the SOA. If a participant withdraws before the end of session 2 their data may still be used for analysis, depending on how much data is available. If there is insufficient data for analysis, at the discretion of the study team, this participant will be replaced.

The end of the study is defined as the date of the last visit of the last participant in the study, e.g. the second follow up session for the last participant.

5.4. Scientific Rationale for Study Design

These challenges provide a method to demonstrate pharmacodynamic effects of novel therapeutics that modulate systemic and local inflammation by measurement of inflammatory mediator production, leukocyte numbers, immune cell phenotypes and opportunity for *ex vivo* assays. The combination of blister induction after GM-CSF or LPS challenge provides a paradigm to investigate chemotaxis and the ability to recognise phenotype switching in mononuclear myeloid cells as local inflammation is thought to be exacerbated by systemic inflammation.

5.5. Dose Justification

5.5.1. Cantharidin

In many studies employing this model (Day, 2001, Morris, 2009, Landis, 2010, Dinh, 2011) [GlaxoSmithKline Document Number (GM2008/00294/00)], 25 µL of 0.1% cantharidin in acetone has been used to induce blisters. Skin blister induction with 0.1% cantharidin solution is generally well tolerated and safe, with side-effects limited to transient hyperaesthesia, discomfort upon pressure and occasional transient hyperpigmentation (Day, 2001). In a previous GSK study [GlaxoSmithKline Document Number (GM2008/00294/00)] there were no significant adverse events attributed to application of 25 µL of 0.1% cantharidin in acetone solution, via soaking a 7-8 mm diameter filter disc, which was held in place in contact with the skin for up to 40 hours. The model was well tolerated in healthy male volunteers.

In a more recent study [GlaxoSmithKline Document Number: (AM2010/00011/01)] the application of cantharidin was refined to produce smaller, more consistently sized blisters. This was achieved by omitting the filter disc and directly applying a lower volume (5 µL) of 0.2 % solution to an area of participants' skin delimited by a small ring of Vaseline (approximately 5-6 mm in diameter). Blisters were harvested up to 72 hours after application of cantharidin. This usually resulted in blisters of up to 10 mm in diameter containing between 100 – 500 µL of fluid. In this way the amount of cantharidin used per blister was both reduced and standardised, with a large resultant decrease in observed variability of blister volume (both within (79% to 31%) and between (94% to 44%) participants). This dose represents a 2.5-fold reduction in the amount of cantharidin used per blister, compared to most previous blister studies. It is important to note that these blister studies themselves had used cantharidin at 1/7 of the concentration recommended for direct skin application for treatment of warts and *Molluscum contagiosum* in children, using multiple simultaneous applications (Noda-Cabrera, Martín et al. 2015). The developed methodology thus represents a substantial reduction in exposure compared to that recommended for therapeutic use; it is the dose and method of application that will be used for all blister induction in this study. This new methodology was well tolerated by healthy male volunteers.

5.5.2. LPS

The initial dose level for LPS is proposed to be 0.5 ng/kg (bioactivity of proposed batch LPS is 6 EU / ng, therefore a dose of 3.0 EU / kg). This dose is associated with a measurable systemic cytokine response but is well tolerated based on the literature. Participants will be hydrated prior to administration of LPS with normal saline at a rate of 250 mL/hr for 4 hours prior to dosing and 8 hours after dosing.

If dose escalation is required to achieve a robust (well tolerated) systemic cytokine response, then this will proceed in increments not exceeding a doubling of the previous dose; the highest permitted dose will be 4 ng/kg. (i.e. 24 EU/kg). Dose escalation will be guided by 1) safety and tolerability 2) the systemic cytokine response although other secondary and exploratory markers may also be taken into account. The goal is to achieve informative biomarker excursions with absent or trivial symptoms experienced by participants.

5.5.3. GM-CSF

Recombinant human GM-CSF (Sargramostim) is FDA approved for the reconstitution of myeloid cells after bone marrow transplantation, neutrophil recovery following chemotherapy in AML patients or mobilisation of peripheral blood progenitor cells, with a recommended dose of 250 μg/m^2/day. GM-CSF is well tolerated in healthy (van Pelt, 1996) and immuno-compromised participants (Lieschke, 1989, Gianni, 1990, Mehta, 2015). A wide range of doses have been administered to oncology patients (0.3 – 30 μg/kg/day) over several consecutive days and whilst are generally well tolerated, with adverse effects including bone pain, myalgia and rash at 15 μg / kg / day. Pericarditis was a dose-limiting toxicity where GM-CSF doses exceeded 15 μg / kg / day (Lieschke, 1989).

A GM-CSF dose of 5 μg / kg, administered subcutaneously, is associated with a measurable leukocyte response but is well tolerated based on the literature. 1.5 μg/kg dose infused intravenously over 2 hours provides the same exposure (AUC) due to the increased bioavailability and higher Cmax concentrations [Cebon, 1990]. For an average male body surface area of 1.9m^2 this equates to a dose of approximately 60μg/m^2, and to remain consistent with clinical practice in dosing GM-CSF therapeutically the 60μg/m^2 will be the starting dose.

If dose escalation is required to achieve a robust (well tolerated) leukocyte response then this will proceed in increments up to a maximum dose of 480 μg/m^2 . Dose escalation will be guided by 1) safety and tolerability 2) the systemic leukocyte response although other secondary and exploratory markers may also be taken into account. The goal is to achieve informative biomarker excursions with absent or trivial symptoms experienced by participants.

6. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participant must be 18 to 45 years of age inclusive, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Participants who are overtly healthy as determined by medical evaluation including: medical history, physical examination, laboratory tests, and ECG.

Weight

3. Body mass index (BMI) within the range 19.0-30.0 kg/m^2 (inclusive).

Sex

4. Male

All participants must agree to use contraception as detailed in Appendix 5 of this protocol during session 2 and refrain from donating sperm from session 2 to end of study (follow-up 2 visit).

Informed Consent

Capable of giving signed informed consent as described in Appendix 3 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.2. Exclusion Criteria

A participant will not be eligible for inclusion in this study if any of the following criteria apply:

1. A positive pre-study Hepatitis B surface antigen or positive Hepatitis C antibody result within 3 months of screening
2. A positive test for HIV antibody.
3. Persistent abnormal CRP/ WCC levels at screening.
4. Abnormal liver function tests at screening. For healthy participants: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and bilirubin ≥ 1.5xupper limit of normal (ULN) (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%) at screening (Refer to Appendix 1).
5. A positive pre-study drug/alcohol screen.
6. Current, or chronic history of:

- liver disease or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones), anaphylaxis, and /or anaphylactoid (resembling anaphylaxis) reactions (Sampson, 2006).
- cardiac, respiratory or renal disease (childhood asthma can be included).
- Sensitivity or severe allergic responses to any of the challenge agents or cantharidin, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator or GSK Medical Monitor, contraindicates their participation.
- vasovagal syncope
- surgery or significant trauma in 3 months leading to study enrolment
- relevant skin conditions (e.g. recent h/o eczema or recurrent eczema, keloid, skin allergies, psoriasis, atopic dermatitis, and vitiligo) which in the opinion of the investigator could pose safety issues or cause interference with study procedures.
- sepsis or known coagulation disorders
- peripheral oedema, lymphangitis, lymphoedema, pleural or pericardial effusion.
- respiratory conditions including but not limited to asthma, COPD, and bronchiectasis and any current respiratory infection.

7. Presence on either forearm of tattoos, naevi, hypertrophic scars, keloids, hyper– or hypo- pigmentation. Participants with very fair skin, very dark skin, excessive hair or any skin abnormalities that may, in the opinion of the Investigator, interfere with study assessments.
8. Unable to refrain from the use of prescription drugs taken on an intermittent (as needed) basis or non-prescription drugs; these include NSAIDs, vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to Day 1 of session 1 and continuing until the final follow up visit).
9. The participant has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 90 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer) or currently in a study of an investigational device.
10. Previous exposure to LPS in a clinical research setting.

- Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56-day period.
- Current smoker or former regular smoker within 6 months before the screening visit.
- Unwillingness or inability to follow the procedures outlined in the protocol.

6.3. Lifestyle Restrictions

6.3.1. Caffeine and Alcohol

- During each session, participants will abstain from caffeine and alcohol for 24 hours before the start of procedures until after collection of the final pharmacodynamics sample.

6.3.2. Activity

- Participants will abstain from strenuous exercise for 48 hours before each session. Participants may participate in light recreational activities during studies (e.g., watching television, reading), but should avoid:
    - Strenuous exercise to the upper limbs whilst blister in place
    - Getting the blister dressing wet during bathing
    - Sunbathing (or sun-bed) on the forearms during the study
    - Topical application of any creams to the forearms for the duration of the study

6.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened.

7. TREATMENTS

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

7.1. Treatments Administered

Study Treatment Name:	Cantharone (Cantharidin)	LPS	GM-CSF	Intravenous Hydration with saline solution (for LPS only)
Dosage formulation:	Liquid (mixture) Ether (42.8 % W/V), Acetone (36.0 % V/V), Alcohol (14.2 % W/V), Camphor (1.2 % W/V), Cantharidin (0.7 % W/V) Balance (5.1%W/V) mixture of pyroxylin and castor oil	LPS is lyophilized in a 1 microgram vial, formulated in 1% lactose and 0.1% PEG6000	The vial of lyophilized LEUKINE contains 250 mcg (1.4×106 IU/vial) sargramostim. The reconstituted lyophilized LEUKINE vial also contain 40 mg/mL mannitol, USP; 10 mg/mL sucrose, NF; and 1.2 mg/mL tromethamine, USP, as excipients	0.9% Sodium Chloride IV bags
Unit dose strength(s)/Dosage level(s):	0.7% cantharidin liquid which will be diluted with acetone to 0.2%	0.5 ng/kg (6 EU/ng) with possible escalation up to 4ng/kg if participants asymptomatic and no cytokine response observed.	60µg/m^2 to a maximum of 480 µg/m^2	1L
Route of Administration	Topical	IV	IV infusion	Intravenous

Study Treatment Name:	**Cantharone (Cantharidin)**	**LPS**	**GM-CSF**	**Intravenous Hydration with saline solution (for LPS only)**
Dosing instructions:	Apply 5 µl of 0.2 % Cantharidin solution (diluted in acetone) directly onto skin in area of ~ 1 cm^2	IV injection of 0.5 ng/kg (6 EU/ng) body weight formulated as suspension in normal saline (or other LPS dose following escalation, see above). LPS will be provided in a final volume of 10 mL normal saline in a 10 mL syringe, and administered over 1-2 minutes.	Dose will be calculated by calculating body surface area using the Mosteller formula to give IV infusion dose in µg/m^2 given over approximately 2 hours	Administer intravenously at a rate of 250 mL/hr for 4 hours prior to dosing with LPS and 8 hours after dosing with LPS
Packaging and Labeling	N/A	N/A	N/A	N/A

Study Treatment Name:	Cantharone (Cantharidin)	LPS	GM-CSF	Intravenous Hydration with saline solution (for LPS only)
Manufacturer	Dormer Laboratories Inc. ADDRESS: 91 Kelfield St. # 5 Rexdale Ontario Canada M9W 5A3	List Biological Laboratories, INC 540 Division Street, Campbell California 95008-6906 USA	Leukine is a registered trademark licensed to Genzyme Corporation. Manufactured by: sanofi-aventis U.S. LLC Bridgewater, NJ 08807 A SANOFI COMPANY US License No. 1752 © April 2013 sanofi-aventis U.S. LLC Phone:PPD PPD	Baxter Inc. Caxton Way Thetford IP24 3SE

7.2. Method of Treatment Assignment

Participants will each have 4 blisters induced in session 1, and 4 blisters in session 2. All blisters will be within the central 60 % of the volar forearm surface. 2 blisters will be induced on each forearm in each session. Blisters induced in the second session will be in the same area of the forearm, but at least 2 cm away from a previous blister site.

In session 2, participants will be randomised (centrally) to have either a LPS or GM-CSF *in vivo* challenge, in a 1:1 ratio in accordance with a randomisation schedule generated by GSK Clinical statistics, prior to the start of the study, using a validated internal randomisation software. Randomisation cannot be reassigned.

7.3. Preparation/Handling/Storage/Accountability

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance.

The investigator or the head of the medical institution, or designated site staff (e.g., storage manager, where applicable) must maintain study treatment accountability records throughout the course of the study.

7.3.1. Cantharidin

Cantharidin is not expected to pose significant occupational safety risk to site staff under proposed conditions of use and administration. Adequate precautions will be taken to avoid direct eye or skin contact and the generation of aerosols or mists. Precaution will be taken to avoid direct contact with the challenge agent. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions will be provided to the investigator. In the case of unintentional occupational exposure, the monitor, medical monitor and/or study manager will be notified.

One part of Cantharone will be mixed with 2.5 parts of pharmaceutical grade acetone in the CUC pharmacy according to standard practice. This reconstituted challenge agent will be used within 4 hours and any residual material will be discarded according to standard GSK waste-streams.

7.3.2. LPS

LPS is not expected to pose significant occupational safety risk to site staff under the proposed conditions of use and administration. Adequate precautions will be taken to avoid direct eye or skin contact and the generation of aerosols or mists. Precaution will be taken to avoid direct contact with the challenge agent. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions will be provided to the investigator. In the case of unintentional occupational exposure, the monitor, medical monitor and/or study manager will be notified.

The dose of LPS will be calculated according to body weight and infused as a bolus over less than 2 minutes. Any residual material will be discarded according to standard GSK waste-streams.

7.3.3. GM-CSF

GM-CSF is not expected to pose significant occupational safety risk to site staff under the proposed conditions of use and administration. Adequate precautions will be taken to avoid direct eye or skin contact and the generation of aerosols or mists. Precaution will be taken to avoid direct contact with the challenge agent. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions will be provided to the investigator. In the case of unintentional occupational exposure the monitor, medical monitor and/or study manager will be notified.

The dose of GM-CSF will be calculated based on body surface area (Mosteller formula: $BSA = 0.016667 \times \text{Weight (kg)}^{0.5} \times \text{Height (cm)}^{0.5}$) [Mosteller, 1987] and infused over approximately a 2 hour period. Any residual material will be discarded according to standard GSK waste-streams.

7.4. Treatment Compliance

- When the individual dose for a participant is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.
- Participants will be dosed at the site and will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and eCRF. The dose of study treatment and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment.

7.5. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrolment or receives during the study must be recorded along with:

- reason for use
- dates of administration including start and end dates
- dosage information including dose and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Participants must abstain from taking prescription or non-prescription drugs (including paracetamol, NSAIDs, steroids, vitamins and dietary or herbal supplements) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study treatment until completion of the follow-up visit, unless, in the opinion of the investigator and sponsor, the medication will not interfere with the study.

Other concomitant medication may be considered on a case-by-case basis by the investigator in consultation with the Medical Monitor if required.

8. DISCONTINUATION CRITERIA

8.1. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

- Refer to the SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.
- Individual participants may be withdrawn from the study if, in the judgement of the investigator, they have exhibited symptoms in response to LPS or GM-CSF that are exaggerated or unanticipated.

8.2. Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA (Section 2).
- Protocol waivers or exemptions are not allowed
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.
- Samples will be stored securely and made accessible only to scientists contributing to and supporting this research. Clear custodianship responsibilities will be in place at all times and receipt/use/disposal details will be tracked on databases as per GSK internal processes. The samples will be stored for a maximum of 5 years, after which they will be disposed of in accordance with the Human Tissue Authority's Code of Practice.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

- The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1. Adverse Events

The definitions of an AE or SAE can be found in Appendix 4.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the study.

9.1.1. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the time when the ICF is signed by the participants until the follow-up visit at the time points specified in the SoA (Section 2).
- All AEs will be collected from the start of session 1 until the follow-up visit at the time points specified in the SoA (Section 2).
- Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4.

9.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AE and/or SAE. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

9.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.2). Further information on follow-up procedures is given in Appendix 4.

9.1.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information e.g. summary or listing of SAE) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.2. Treatment of Overdose

Cantharidin: Treatment of Challenge Agent Overdose

- Participants will not have access to cantharidin and overdose is therefore extremely unlikely. In the event of overdose, the clinical management will be based on symptomatic treatment and supportive measures as indicated and required according to current UK guidelines.

- In case of any overexposure to Cantharone refer to Material Safety Data Sheet (MSDS).

- Inhalation: Remove victim to fresh air. Give oxygen or artificial respiration if necessary.

- Skin Contact: Immediately flood affected skin with water while removing and isolating all contaminated clothing. Gently wash all affected skin areas thoroughly with soap and water. Seek medical attention if warranted.

- Eye Contact: First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control centre. Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. Immediately transport the victim after flushing eyes to a hospital even if no symptoms (such as redness or irritation) develop.

- Ingestion: Do not induce vomiting. If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and immediately call a hospital or poison control centre. Immediately transport the victim to a hospital. If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open, and lay the victim on his/her side with the head lower than the body. Transport the victim immediately to a hospital.

LPS: Treatment of Challenge Agent Overdose

- LPS dose selection has been made on the basis of a lower dose and potency than is usually reported in the literature, and thus overdose is not anticipated.
- Intravenous LPS has the potential to be extremely harmful in overdose and induce symptoms and organ dysfunction in keeping with septic shock. Participants who receive an overdose of intravenous LPS will be hydrated with rapid intravenous fluid boluses. Clinical staff may need to expedite immediate transfer to hospital in the event that volunteers exhibit clinical signs of septic shock. Mild pyrexia and malaise may be observed.

GM-CSF: Treatment of Challenge Agent Overdose

- In case of overdose, participant will be carefully monitored for WBC increase and respiratory symptoms. Symptomatic management will be carried out according to existing guidelines.

9.3. Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

9.3.1. Height and body weight

Height and body weight will be measured and recorded in PIMS. Body weight at session 2 Day -1 will be used to calculate the dose of LPS and GM-CSF to be used.

9.3.2. Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the skin, cardiovascular, respiratory, gastrointestinal and neurological systems.
- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system and abdomen (liver and spleen).

9.3.3. Assessment of blister sites

- Visual assessment of blister site will be carried out at selected visits.

9.3.4. Vital Signs

- Vital sign measurements will include systolic and diastolic blood pressure, respiratory rate, SpO2 and pulse rate.
- Blood pressure and pulse measurements will be assessed in a semi-supine position with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting in a semi supine position without distractions (e.g., television, cell phones).
- Vital signs (to be taken before blood collection for laboratory tests at times detailed in SoA) will consist of 1 pulse and 1 blood pressure measurement.
- Temperature will also be taken

9.3.5. Electrocardiograms

- Triplicate 12-lead ECG will be obtained at screening and baseline as outlined in the SoA using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. At all other time points a single ECG measurement will be taken.
- All scheduled time points for ECG measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting in a semi supine position without distractions (e.g., television, cell phones).
- At each time point at which triplicate ECG are required, 3 individual ECG tracings will be obtained as closely as possible in succession.
- Participants dosed with LPS, will receive telemetry for a minimum of 6 hours post-dose or until their telemetry shows no clinical significant findings for 4 hours (whichever is longer).
- Participants undergoing the GM-CSF challenge may be connected to continuous cardiac telemetry during their stay in the unit at the discretion of the investigator if they are symptomatic.

9.3.6. Participant Diary Card

Participants will be given a diary card for them to record the healing time of the blisters as well as to record any adverse events or medications taken whilst outside of the unit.

9.3.7. Pain scale

After the final blister sample in each session is taken, participants will be asked to rate their level of pain from blister induction on an 11-point scale (0-10), with 0 being "no pain" and 10 being "worst pain imaginable" (Farrar, 2001).

9.3.8. Clinical Safety Laboratory Assessments

- Refer to Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 7 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal by the investigator or medical monitor.
- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the aetiology should be identified and the sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the laboratory manual and the SoA.

9.4. Genetics

Genetics are not evaluated in this study. Transcriptome analysis will utilise Next Generation Sequencing (NGS) methods, which are described in Section 9.7.1.4.

9.5. Pharmacokinetics

Pharmacokinetics are not evaluated in this study.

9.6. Pharmacodynamics

Some of the pharmacodynamic endpoints may be reported separately from the main clinical study report.

9.7. Biomarkers

There will be several different types of biomarker samples collected during the study, as outlined in the SOA, including biomarkers from blood, urine and blister fluid. Some of the exploratory biomarkers may be reported separately from the main clinical study report.

9.7.1. Biomarkers in blood

9.7.1.1. Mediators blood sample

Approximately 6 mL of blood will be collected into SST or Na Heparin tubes according to the timings on the SoA. This will allow for analysis of serum (or plasma) to measure inflammatory mediators. Methods of analysis which may be used on these samples are detailed in the Study Reference Manual. Measurement of soluble inflammatory mediators will be carried out in batch analysis.

9.7.1.2. Flow cytometry blood sample

Blood samples of approximately 2 mL will be collected into SST or Na Heparin tubes for measurement of leukocyte number and activation markers by flow cytometry (see indicative list in endpoints), at times specified in the SoA.

9.7.1.3. *Ex vivo* stimulation blood sample

For the LPS challenge participants only, 2x 1 mL whole blood samples will be collected at pre-dose and 6 hours post-LPS dose. These samples will be drawn into TruCulture tubes containing LPS and the other in a TruCulture null tube, and incubated for 24 hours. Cellular and soluble contents will be separated and inflammatory mediators analysed in the soluble fraction.

9.7.1.4. Transcriptome blood sample

In session 2, whole blood will be collected into PAXgene tubes for transcriptome analysis. Blood (2.5 ml) will be collected prior and several time points post LPS or GM-CSF challenge as detailed in SoA. Samples will be used to extract RNA and prepare cDNA which will subsequently be used for transcriptome analysis by Next Generation Sequencing (NGS) methods. NGS data will be aligned and mapped to human genome reference sequences and analysed for differential expression for each challenge type.

9.7.2. Biomarkers in urine

The pre-challenge urine sample will be collected during session 1. The post-challenge urine samples will be collected during session 2 after LPS challenge. In session 2, participants will be encouraged to pass urine immediately before LPS challenge dose and urine voids will be collected from after LPS until 12 hours post-LPS and the time of the urine collection will be recorded. These samples will be collected for measurement of tetranor-PGDM and other inflammatory mediators. Tetranor-PGDM is the stable urinary metabolite of the prostaglandin PGD2. While tetranor-PGDM has very low natural abundance in normal healthy individuals, it has been shown to be elevated in the urine of patients with Duchenne muscular dystrophy. Elevation of urinary tetranor-PGDM in normal healthy volunteers through LPS challenge would allow for evaluation of target engagement in that population Tetranor-PGDM and related metabolite will be measure by mass spectroscopy as samples are collected.

9.7.3. Biomarkers in blisters

Biomarkers in blisters will be sampled at 24 and 48 hours post blister induction and collected in polypropylene micro-centrifuge tubes. Cellular and soluble contents will be separated. The volume of the blister sample will be calculated and recorded in the eCRF.

9.7.3.1. Flow cytometry blister sample

Blister sample will be used to measure leukocyte number and activation markers by flow cytometry (see indicative list in endpoints), at times specified in the SoA.

9.7.3.2. Mediators blister sample

The soluble fraction from the blister sample will be used to measure inflammatory mediators. Methods of analysis which may be used on these samples are detailed in the Study Reference Manual.

9.8. Health Economics OR Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

10. STATISTICAL CONSIDERATIONS

This is an exploratory enabling study which is primarily designed to estimate the effect of systemic exposure to LPS or GM-CSF challenge on soluble and cellular inflammatory biomarkers in cantharidin-induced skin blister model. There are no formal hypotheses to be tested.

10.1. Sample Size Determination

Participants will be randomised into the study, in a 1:1 randomisation ratio to either LPS or GM-CSF challenge. It is estimated that up to 16 participants may be evaluated at the optimal dose (8 participants for each challenge agent).

- Part I (up to 28 participants): Up to 6 cohorts of 4 participants (2 for each challenge) may be enrolled. A further 4 participants (2 for each challenge) will be enrolled into the cohort at the optimal dose selected.
- Part II (up to 8 participants): Based on the decisions from an interim analysis following Part I of the study, an additional cohort of up to 8 participants (4 for each challenge) may be enrolled into the cohort at the optimal dose selected.
- For Part I and II (up to 4 participants), if participants prematurely discontinue the study, additional replacement participants may be recruited. A 10% drop-out rate has been assumed.

These numbers have been determined partly through feasibility but considerations has also been given for this study to generate data that can provide the foundation for future

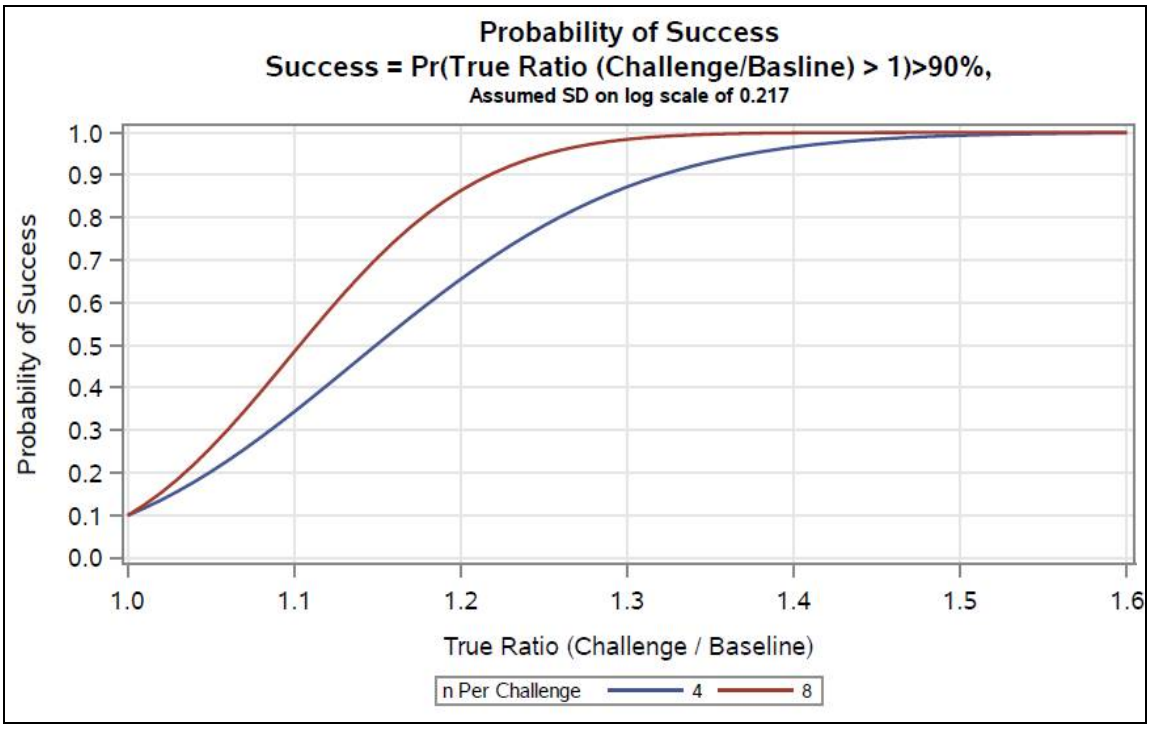
studies testing the effect of compounds that target key pathways in inflammatory and autoimmunity. The 2-stage design provides an efficient way to adapt the sample-size, based on emerging data from Part I, so that estimates of response and variability of primary endpoints can be generated.

Following assessment of past studies from literature and GSK, no direct estimates of primary endpoints based on LPS and GM-CSF challenges within a cantharidin-induced skin blister model were reported. However, estimates of inflammatory response for circulating TNF-α and IL-6 which may be translatable to those observed in this study at the optimal dose selected have been reported, albeit with some degree of uncertainty :

- Estimates for circulating urinary tetranor PGDM and total leukocyte numbers are very limited and Part I of the study aims to generate in-house estimates to inform sample size.
- It is assumed for the purposes of sample size calculation, that the between subject standard deviation on the $\log_e$ scale for TNF-α and IL-6 are 0.217 and 0.394 respectively (GSK study EMI114416). These estimates reflect the variability of cytokines within a cantharidin-induced skin blister model, albeit in the absence of challenge agent.
- Based on published data (Suffredini, 1999, Dillingh, 2014, Ferguson, 2014, Kiers, 2017) fold increases from baseline were observed in the range of 42 to 200 for TNF-α and 100 to 300 for IL-6 using an IV 1 ng/kg (10EU/ng) LPS challenge. Variability was reported with limited estimates to be directly used for sample size calculations.

Using an estimate of SD the $\log_e$ scale = 0.217 (EM114416) for TNF-α and a sample size of 4 and 8 participants, the probability that the true ratio between systemic challenge and baseline being greater than 1 can be calculated for a range of unknown true differences. . 3 below shows the probability of success for the stated assumptions, for a range of true treatment ratios.

Figure 3 Probability of Success for Cytokines



For example, the probability of achieving study success (i.e. based on observed fold increases from baseline in literature and therefore representing robust inflammatory cytokine responses with the challenge) assuming the true increase of cytokines with challenge agent over baseline is 40% (ratio of 1.4) is greater than 90% (for n=4 and 8 participants). If the truth is that there is no increase over baseline (ratio=1) then there is a 10% chance of incorrectly declaring success.

Sample Size Sensitivity

Table 1, provides the probability of achieving study success assuming the true increase of cytokines with challenge agent over baseline is 40% (ratio of 1.4) and assuming larger variability is observed.

Table 1 Probability of Success for Cytokines Assuming Higher Variability Observed

Assumed SD (Loge scale)	True Ratio (Challenge/Baseline)	Probability of Success (%)	
		n = 4	n = 8
0.394 [1]	1.4	66%	87%
0.434 [2]	1.4	61%	82%
0.473 [3]	1.4	56%	77%

[1] Higher variability observed in study EMI114416; [2] Increase of 10% for SD=0.394; [3] Increase of 20% for SD=0.3984

Sample Size Re-estimation

Sample size re-estimation will be conducted following Part I of the study. If there is an increase in the precision of estimates for inflammatory responses, then additional participants will be enrolled.

10.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Screened	All participants who were screened for eligibility
Enrolled	All participants who sign the ICF
Randomized	All participants who are randomized to receive and are given the treatment (LPS or GM-CSF challenge)
Evaluable	All participants who complete the study

As defined in Section 5.3, a participant is considered to have completed the study if he/she has completed up to the end of session 2 of the study. The end of the study is defined as the date of the last visit of the last participant in the study, e.g. the second follow up session for the last participant.

10.3. Statistical Analyses

All data will be summarised using descriptive statistics and/or graphical displays and listed.

Endpoint	Statistical Analysis Methods
Primary	For the optimal dose selected, and if sufficient data in available, primary biomarker endpoints will be analysed using a Bayesian repeated measures random effects model. Data maybe transformed (e.g. log) prior to the analysis if warranted. Terms in the model may include: challenge, challenge by time interaction, blister site and forearm. Additional covariates maybe included if deemed appropriate. Adjusted means and 95% credible intervals will be constructed for each of the challenges at each time point in addition to the comparison of systemic challenge with LPS/baseline and GM-CSF/baseline. Specific probabilities that the true difference (i.e. challenge to baseline) is greater than specified quantities may also be produced. Full details of the statistical analysis will be documented in the Reporting and Analysis Plan (RAP).
Secondary	The proposed statistical analyses planned for the primary biomarker endpoints will be repeated for secondary endpoints.

Endpoint	Statistical Analysis Methods
Exploratory	Full details will be described in the RAP.

10.3.1. Interim Analyses

There will be ongoing data reviews conducted by the study team of any available data through the study progression. For Part I, data reviews will be performed by the study team to support whether to dose escalate to the next cohort for each challenge agent. At the end of Part I, an interim analysis will be conducted to decide whether to enrol additional participants in Part II of the study, for the optimum dose selected in Part I.

Full details of the interim analysis will be pre-specified in the RAP.

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

Abbreviations	
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BMI	Body Mass Index
CD	Cluster of Differentiation
cDNA	Complementary DNA
CIOMS	Council for International Organizations of Medical Sciences
Cmax	Maximum Concentration
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic Obstructive Pulmonary Disease
(e)CRF	(electronic) Case Report Form
CRP	C-reactive protein
CSR	Clinical Study Report
CUC	Clinical Unit Cambridge
ECG	Electrocardiogram
GCP	Good Clinical Practice
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GSK	GlaxoSmithKline
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA-DR	Human Leukocyte Antigen - antigen D Related

ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IL	Interleukin
IV	Intravenous
IRB/IEC	Institutional Review Boards /Independent Ethics Committees
LPS	Lipopolysaccharide
MCH	Mean Corpuscular Hemoglobin
MCP	Monocyte chemoattractant protein
MCV	Mean Corpuscular volume
mRNA	Messenger RNA
MSDS	Material Safety Data Sheet
NF-kB	Nuclear Factor kB
NGS	Next Generation Sequencing
NSAIDS	Nonsteroidal anti-inflammatory drugs
PBPC	Peripheral Blood Progenitor Cells
PD	Pharmacodynamics
PGD2	Prostaglandin D2
PGE2	Prostaglandin E2
PR	Pulse rate
PRR	Pattern Recognition Receptors
RAP	Reporting Analysis Plan
RBC	Red Blood Cell
SAE	Serious Adverse Event
SD	Standard Deviation
SoA	Schedule of Activities

SRM	Study Reference Manual
SST	Serum-Separating Tube
SUSAR	Suspected Unexpected Serious Adverse Reactions
TNF	Tumour Necrosis Factor
ULN	Upper limit of normal

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
NONE

Trademarks not owned by the GlaxoSmithKline group of companies
Cantharone
Leukine

12.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in 2 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 2 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet Count RBC Count Hemoglobin Hematocrit PT and APTT (at screening only)	RBC Indices: MCV MCH %Reticulocytes	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	BUN	Potassium	Aspartate Aminotransferase (AST)	Total and direct bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)	Total Protein
	Glucose (fasted glucose at screening only)	Calcium	Alkaline phosphatase	CRP
	Albumin	Urea	Corrected calcium	
Routine Urinalysis	• Urine cotinine • Specific gravity • pH, glucose, protein, blood, ketones by dipstick • Microscopic examination (at investigator discretion if blood or protein urine dipstix is abnormal.)			
Other tests	• HIV • Hepatitis B (HBsAg and HBcAb) • Hepatitis C (Hep C antibody) • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)			

NOTES :

1. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

12.3. Appendix 3: Study Governance Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable ICH Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g. advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study centre.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Participants who are rescreened are required to sign a new ICF.

The ICF may contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research in accordance with SOP-GSKF-410. The investigator or authorized designee will explain to each participant the objectives of the exploratory research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining specimens to be used for exploratory research. Participants who decline to participate will not provide this separate signature.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Committees Structure

A dose escalation committee will review data after each Group (2 participants) following analysis of key primary endpoints to determine a dose which gives a robust inflammatory response.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicentre studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Dissemination of Clinical Study Data

- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study participants, as appropriate.
- A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge.

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g. laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the

currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR) / equivalent summary unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the Source Document Agreement.

Study and Site Closure

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of GSK. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study treatment development

12.4. Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g. ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e. not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE. • The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" constitutes an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g. endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g. hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to** GSK**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed CRF. • The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the Primary Medical monitor. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE reporting can be found in the study reference manual (SRM).

12.5. Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

Contraception Guidance

Male participants

- Male participants with female partners of child-bearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frame in Section 6.1:
    - Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
    - Agree to use a male condom plus an additional method of contraception with a failure rate of <1% per year when having penile-vaginal intercourse with a woman of childbearing potential
- Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration during the protocol-defined time frame.
- In addition, male participants must refrain from donating sperm from session 2 to end of final study follow-up.

Collection of Pregnancy Information

Male participants with partners who become pregnant

- Investigator will attempt to collect pregnancy information on any male participant's female partner of a male study participant who becomes pregnant while participating in this study. This applies only to participants who receive study treatment.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 24 hours of learning of the partner's pregnancy.
- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of foetal status (presence or absence of anomalies) or indication for procedure.